

KU LEUVEN

<i>Acknowledgement</i>	<p><i>Journal of Anxiety Disorders</i></p> <p>This article may not exactly replicate the final published article. It is not the copy of the record.</p>
<i>Archived version</i>	Author manuscript: post-print (post-refereeing)
<i>Published version</i>	http://dx.doi.org/10.1016/j.janxdis.2014.09.006
<i>Journal homepage (Copyright)</i>	http://www.journals.elsevier.com/journal-of-anxiety-disorders/ ISSN: 0887-6185, ESSN: 1873-7897
<i>Author contact at KU Leuven</i>	bert.lenaert@ppw.kuleuven.be

(article begins on next page)

Aversive learning and generalization predict subclinical levels of anxiety:

A six-month longitudinal study

Bert Lenaert

University of Leuven, Belgium

Yannick Boddez

University of Leuven, Belgium

James W. Griffith

Northwestern University, Chicago, IL

Bram Vervliet

University of Leuven, Belgium

Koen Schruers

Maastricht University, Netherlands

Dirk Hermans

University of Leuven, Belgium

Author Note

Bert Lenaert, Yannick Boddez, Bram Vervliet and Dirk Hermans: Centre for the Psychology of Learning and Experimental Psychopathology, University of Leuven, Belgium. James W. Griffith, Department of Medical Social Sciences, Northwestern University, Chicago, IL. Koen Schruers, School for Mental Health and Neuroscience, Maastricht University, Netherlands.

This research was supported by the Center of Excellence on Generalization Research (GRIP*TT; University of Leuven grant PF/10/005). Bert Lenaert is a research assistant for the FWO-Flanders.

Correspondence concerning this article should be addressed to Bert Lenaert, Centre for the Psychology of Learning and Experimental Psychopathology, Faculty of Psychology and Educational Sciences, University of Leuven, Tiensestraat 102 - Bus 3712, 3000 Leuven, Belgium. Email: bert.lenaert@ppw.kuleuven.be

Abstract

The identification of premorbid markers of risk for psychopathology is one of the most important challenges for present-day psychiatric research. This study focuses on behavioral vulnerability factors that contribute to the development of anxiety. Little is known about the role of aversive learning and generalization in the development of pathological anxiety. In this study, a large student sample ($N = 375$) completed a differential aversive learning task followed by a test of generalization. Anxiety was assessed at that moment and after a six-month follow-up. Results showed that both predictors (discrimination learning and generalization) added significantly to the explained variance in anxiety symptomatology at follow-up. These results highlight the importance of longitudinal designs and indicate that screening for individual differences in aversive learning and generalization may foster prediction of anxiety disorders, paving the way for targeted prevention.

Keywords: anxiety, aversive learning, discrimination learning, generalization, longitudinal study

Aversive learning and generalization predict subclinical levels of anxiety:**A six-month longitudinal study**

According to diathesis-stress models, mental disorders arise from the interaction between vulnerability factors (i.e., diatheses) and life stress (e.g., Zvolensky, Kotov, Antipova, & Schmidt, 2005). These vulnerability factors may be characterized at the (neuro-)biological (e.g., anomalies in the amygdala-based fear circuitry in anxiety disorders; Shin & Liberzon, 2010), or behavioral level (e.g., negative attributional style in depression; Jacobs, Reinecke, Gollan, & Kane, 2008; irregularities in smooth pursuit eye tracking in schizophrenia; O'Driscoll & Callahan, 2008). Identifying vulnerability factors allows for accurate prediction and paves the way for targeted prevention (Beauchaine, 2009). The personal and societal costs of mental health problems are immense and predicted to increase (e.g., Murray et al., 2012). Thus, prediction and prevention of psychopathology is a major challenge for psychiatric research (World Health Organization Mental Health Action Plan, 2013). Identifying behavioral markers of psychopathology is particularly relevant. Assessing behavior is minimally invasive for the tested individual and is easily and cheaply applicable by the scientist-practitioner in the clinic. Hence, the detection of behavioral markers of risk for psychopathology provides both a theoretical and practicable answer to the challenges ahead.

The present study focuses on behavioral vulnerability factors for anxiety. One major pathway to anxiety disorders is aversive learning (Lissek et al., 2005; Mineka & Zinbarg, 2006). This is a form of associative learning in which an originally neutral stimulus (conditional stimulus; CS) comes to evoke fear reactions after pairings with an aversive stimulus (unconditional stimulus; US). Fear can be highly adaptive by motivating defensive reactions in the face of danger. Abnormalities in aversive learning, however, may contribute to the development of anxiety disorders (Lissek et al., 2005). These abnormalities may manifest as a deficit in discriminating between signals of danger and signals of safety. For instance, a panic attack bears symptomatic resemblance to a heart attack, but only the latter represents actual danger (Haddad, Pritchett, Lissek,

& Lau, 2012). Thus, *impaired discrimination learning* between safety signals and danger signals may contribute to the development of anxiety symptoms. Deficits in discriminatory aversive learning have been demonstrated in individuals suffering from an anxiety disorder (e.g. Lissek et al. 2009; Grillon & Morgan, 1999), as well as in individuals with subclinical levels of anxiety (e.g. Arnaudova et al., 2013; Chan & Lovibond, 1996; Haddad, Pritchett, Lissek, & Lau, 2012; Gazendam, Kamphuis, & Kindt, 2013; but see also Torrents-Rodas et al., 2013; Indovina, Robbins, Núñez-Elizalde, Dunn, & Bishop, 2011).

Maladaptive consequences of irrational fears are greatly multiplied by the generalization of aversive learning. Generalization occurs when a conditioned response is elicited by a stimulus different from but similar to the actual CS. Generalization reduces the need to rediscover contingencies that have proven to be important in the past (Hermans, Baeyens, & Vervliet, 2013). It becomes pathological, however, when the conditioned fear reactions are frequently elicited in the absence of actual threat. For instance, after a biting incident, an individual may react fearfully to the dog that was involved, but also to other, more or less similar dogs, or even to seeing a dog on television, which obviously represents no imminent threat. In the same logic, individuals who show increased *generalization* may be more prone to develop anxiety complaints. Differences in generalization have been demonstrated in individuals suffering from panic disorder, relative to healthy controls, with the former displaying stronger generalization (Lissek et al., 2010).

In order to test hypotheses about the role of discriminatory aversive learning and generalization in the etiology of anxiety disorders, longitudinal studies are critically needed (Kraemer et al., 1997). The present study is a prospective investigation of the relationship between anxiety and both aversive learning and generalization. This adds significantly to the current set of cross-sectional studies by testing whether the known deficits are consequences or antecedents of anxiety complaints (diagnostic markers or vulnerability factors, respectively; Beckers, Krypotos, Boddez, Effting, & Kindt, 2013). Moreover, the identification of premorbid markers of risk for the development of anxiety complaints requires, by definition, the use of non-clinical samples. The

present study was conducted in a large group of first-year psychology students. Because the transition to university is accompanied by a set of stressors related to academics, finances, social interaction, and other issues, students are particularly interesting from a diathesis-stress perspective (Dyson & Renk, 2006; Gefen, 2010).

At a baseline assessment, we measured levels of self-reported anxiety and administered a differential aversive learning task followed by a test of generalization to all participants. After a six month follow-up period, participants completed the anxiety measures again. Most cross-sectional studies have used individually selected levels of electrical stimulation on the arm as aversive US. For practical applicability purposes, we decided to use individually selected levels of aversive pictures of the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2008). Also, we focused on simple verbal ratings as measures of aversive learning and generalization, instead of complicated psychophysiological measurements. These decisions ensure easy use and wide applicability in out-of-laboratory settings, which is critical for implementation of risk detectors.

The aversive learning phase consisted of two circles differing in size (Lissek et al., 2010), one of which (CS+) was contingently followed by negative emotional pictures (US). The other circle (CS-) was never followed by the US. The dependent variables were verbal US-expectancy and fear ratings that were collected during each circle presentation. We hypothesized that impaired differentiation in US-expectancy between the two circles would predict higher levels of anxiety six months later (statistically controlling for baseline anxiety). Following the differential aversive learning phase, various circles of different sizes between the CS+ and the CS- (generalization stimuli; GSs) were tested for their ability to elicit US-expectancy and fear ratings. We hypothesized that increased generalization would predict higher levels of anxiety six months later (statistically controlling for baseline anxiety).

Method

Participants

Participants were 375 first year psychology students at the University of Leuven, Belgium, who completed the differential aversive learning task and test for generalization. Twenty students could not be invited for follow-up because they failed to provide their anonymous ID before completing the questionnaires. Therefore, the analyses of Time 1 (T1) are based on 355 participants (288 women). Their mean age was 18.3 ($SD = 1.2$, range: 17-29). At Time 2 (T2), six months later, 273 participants (231 women) of the original 355 (77%) completed the follow-up questionnaires. All participants gave informed consent and received course credits for their participation.

Apparatus and stimuli

Stimuli were presented on a Dell desktop computer screen (48.3 cm). The stimulus sequence, the presentation of the stimuli, and the inter-trial intervals were controlled by Affect 4.0 (Spruyt, Clarysse, Vansteenwegen, Baeyens, & Hermans, 2010).

Based on the Lissek et al. (2010) conditioned generalization paradigm, two circles served as the conditional stimuli. For half of the participants, a small circle (5.08 cm) was the CS+, and a large circle (11.94 cm) was the CS-. For the other half, this was reversed. The generalization stimuli (GSs) were eight circles, with each GS increasing 15% in diameter starting from the smallest circle, thus creating a continuum of perceptual change between the smallest circle and the largest circle. The diameters, in centimeters, of the eight GSs were 5.84, 6.6, 7.37, 8.13, 8.89, 9.65, 10.41, and 11.18 respectively.

The unconditional stimuli (US) were pictures from the International Affective Picture System (Lang, Bradley, & Cuthbert, 2008). Nine pictures were selected based on previously obtained arousal ratings in young adults (Grühn & Scheibe, 2008). Importantly, these nine pictures were divided into three categories that were created to obtain three different levels of US-aversiveness. In conditioning paradigms that employ an electrical stimulus as US, the intensity of the aversive stimulation during the experiment is usually not fixed, but instead chosen by

participants before the start of the experiment in order to find a level of stimulation that is rated as highly uncomfortable but not painful. Likewise, in this experiment, the three categories of USs differed in aversiveness, allowing participants to choose between mild, moderate, or severe US aversiveness. The most aversive USs were pictures of a thoracotomy, a bloodied corpse and an aggressive dog, with a mean arousal rating of 7.4. The moderately aversive USs depicted a tribal mutilation, fecal matter, and a hospitalized infant, with a mean arousal rating of 5.9. The mild USs depicted a firearm, a cockroach, and a paper bag of vomit, with a mean arousal rating of 5.1.¹ All stimuli were presented on a white background in the center of the computer screen.

The experimental trials consisted of the presentation of a circle that was (in the case of a CS+) or was not (in the case of a CS- or a GS) followed by a US. The US was presented for 1000ms immediately after termination of the CS+. The mean inter-trial interval was 2500ms and ranged from 2300ms to 2700ms.

Measures

US-expectancy and fear ratings. On each trial, participants rated, using a 0-10 scale, their expectancy that the circle (CS+, CS-, or GS) would be followed by a picture. The anchors for the scale were '0' meaning 'I am absolutely sure that no picture will follow' and '10' meaning 'I am absolutely sure that a picture will follow'. Participants selected their rating by placing a red dot across the scale, using the left and right arrows on a standard keyboard. The expectancy score was confirmed by pushing the 'Enter' key. There was no time limit for this response. At the end of each phase of the experiment, participants indicated, using a 0-10 scale, the amount of fear that was evoked by the CS+ and the CS- with '0' meaning 'not at all fear-evoking' and '10' meaning 'very fear-evoking'.

Anxiety questionnaires

Depression Anxiety Stress Scales (DASS-21; Lovibond & Lovibond, 1995). The DASS-21 is the short version of the standard 42-item DASS (Dutch translation by de Beurs, Van Dyck, Marquenie, Lange, & Blonk, 2001). It consists of three subscales with scores ranging from 0 to 21.

Each subscale measures a negative emotional state during the previous week: depression (DASS-D), anxiety (DASS-A), and stress (DASS-S). In past studies, Cronbach's alphas have been adequate: .88 for the Depression scale, .82 for the Anxiety scale, and .90 for the Stress scale (Henry & Crawford, 2005). The DASS-A subscale allows measuring anxiety symptoms isolated from stress and depression, focusing primarily on somatic sensations of anxiety and worry about the loss of control (Brown, Chorpita, Korotitsch, & Barlow, 1997). The DASS-21 also has good discriminant validity of anxiety versus depression (Henry & Crawford, 2005). Because we investigated anxiety specifically, our hypotheses were restricted to the DASS-A subscale.

State and Trait Anxiety Inventory (STAI; Spielberger, 1983). The STAI measures state (STAI-S) and trait anxiety (STAI-T) on two separate subscales, both ranging from 20 to 80, with higher scores indicating higher levels of anxiety. The Dutch version by van der Ploeg (2000) was used, which has good reliability and validity.

Procedure

The experiment took place in a classroom equipped with Dell desktop computers, where participants were invited in groups of 25 or fewer. At the beginning of the experiment, participants gave their informed consent. All participants received written instructions to work in silence at their own pace. First, the questionnaires were administered online using specialized software (www.surveymonkey.com). Then, participants received the instructions that pictures with a potentially negative emotional content would be presented. At this point, the three US-aversiveness levels were introduced by describing the content of the pictures (e.g. "The pictures show the bloodied head of a human corpse"). Participants were requested to choose a US-aversiveness level that they judged to be unpleasant, but not unbearable.

Subsequently, the aversive learning task began. Participants were informed that a number of circles would be presented on the computer screen, some of which would be followed by a picture from the set they had selected. They were explicitly instructed to determine which circles were followed by a picture.

The aversive learning paradigm consisted of three phases. During the first, pre-acquisition phase, the CS+ and the CS- were both presented three times in random order, all in the absence of a US. During the second, acquisition phase, one CS (CS-) was presented 12 times and was never followed by a US. The other CS (CS+), also presented 12 times, was followed by a US nine times (three presentations of each of three pictures in the chosen US-intensity category). Trials in this phase were presented in random order. In the third phase, which was the test for generalization, two CS+ trials, two CS- trials, and one trial for each of the eight GSs were presented in random order. In this phase, one of the two CS+ trials was followed by a US in order to prevent extinction.

Data analysis

The data from the aversive learning task were analyzed using repeated measures analysis of variance (ANOVA). Importantly, based on Lissek et al. (2010), the eight GSs that were presented during the test for generalization were grouped together in four generalization classes, each class representing the average of two neighboring circles. The two CS+ trials and the two CS- trials presented during this phase were grouped together in the analyses as well, thus obtaining a total of six stimulus classes (CS+, C1, C2, C3, C4, CS-), with C1 containing the two GSs most similar to the CS+, and C4 containing the two GSs most similar to the CS-.

Hierarchical regression analysis was used to test whether individual differences in discrimination learning and generalization predicted levels of anxiety measured six months later, using baseline anxiety as a covariate. In order to obtain an index of discrimination learning for each participant, the summed US-expectancy ratings of the last three CS- trials of acquisition were subtracted from the summed US-expectancy ratings of the last three CS+ trials. This total was then divided by three, resulting in an index on a scale from 0 to 10 that reflects the extent to which participants learned to discriminate between the CS+ and the CS- by the end of acquisition.

Results

Aversive learning task

Participant characteristics

Among the 355 participants of whom all T1 data were available, the majority chose the most aversive USs ($n = 190$; 53.5%). The moderately aversive USs were chosen by 148 participants (41.7%), whereas only 17 participants (4.8%) chose the mildest level of US-aversiveness. There were no significant differences in performance on the aversive learning task as a function of the chosen US-aversiveness. Thus, we report analyses for the sample as a whole.

Differential aversive learning task

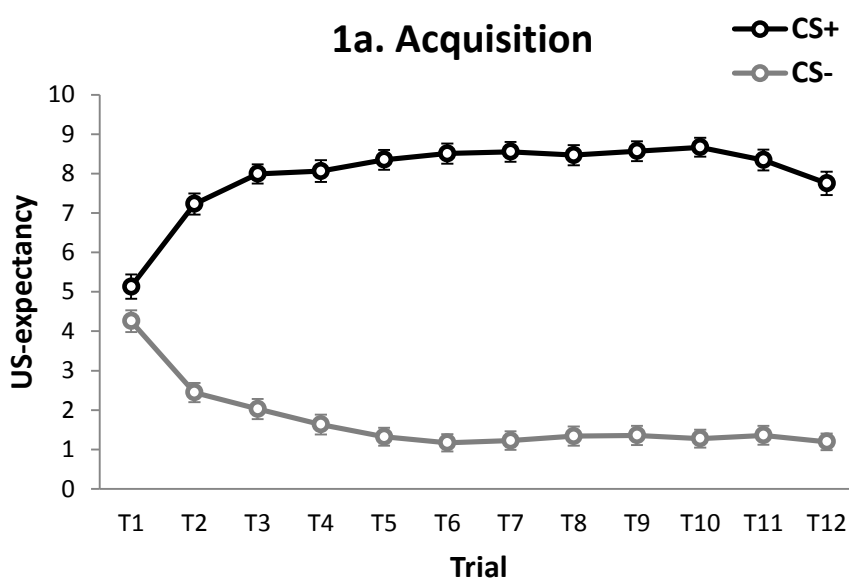
Overall, participants clearly acquired the discrimination between the CS+ and the CS-. This was evidenced by higher US-expectancy ratings to the CS+ than to the CS- at the end of acquisition as compared to the start of acquisition (*Fig. 1a*). At the first trial, the mean US-expectancy ratings were 5.1 for the CS+, and 4.3 for the CS-. At trial 12, this was 7.7 for the CS+, and 1.2 for the CS-. A repeated measures ANOVA with CS-type (CS+ and CS-) and Trial (trial 1 - trial 12) as within-subject variables revealed an effect of CS-type, $F(1,354) = 2173.6$, $p < .001$, partial $\eta^2 = .86$, and a CS-type \times Trial interaction, $F(11, 3894) = 126.41$, $p < .001$, partial $\eta^2 = .26$. Subsequent comparisons revealed a clearly present differentiation between CS+ and CS- at the end of acquisition, $F(1,354) = 1118.08$, $p < .001$, partial $\eta^2 = .76$. Moreover, from trial 1 to trial 12 there was an increase in US-expectancy ratings for the CS+, $F(1,354) = 144.86$, $p < .001$, partial $\eta^2 = .29$, as well as a decrease in ratings for the CS-, $F(1,354) = 346.07$, $p < .001$, partial $\eta^2 = .49$. At the first trial, however, the difference between the CS+ and CS- was already significant, $F(1,354) = 13.56$, $p < .001$, partial $\eta^2 = .04$. Analysis of the pre-acquisition data revealed that the expectancy of the US was highest for the largest circle prior to acquisition, supporting the need for counterbalancing. Because the counterbalancing was executed at the level of testing sessions rather than at the participant level, the largest circle served as the CS+ for 194 participants, whereas the smallest circle served as the CS+ for another 161 participants. Hence, the significant difference between

CS+ and CS- on the first acquisition trial is most probably due to the larger proportion of participants for whom the largest circle served as the CS+. Before the start of acquisition, verbal fear ratings were given for both the CS+ and the CS-, averaging to 3.3 and 2.8 respectively. At the end of acquisition, this was 6.0 for the CS+ and 1.1 for the CS-. A repeated measure ANOVA with CS-type (CS+ and CS-) and Trial (pre-acquisition and post-acquisition) as within-subject variables revealed a significant effect of CS-type, $F(1,354) = 23.75$, $p < .001$, partial $\eta^2 = .06$, and a significant CS-type \times Trial interaction, $F(1,354) = 280.36$, $p < .001$, partial $\eta^2 = .44$. Importantly, subsequent comparisons revealed a significant increase in fear ratings for the CS+ from pre-acquisition to post-acquisition, evidencing that this paradigm was successful in aversive learning, $F(1,354) = 218.86$, $p < .001$, partial $\eta^2 = .38$.

Finally, the index of discrimination learning that was calculated for the last three CS+ trials and the last three CS- trials revealed a mean discrimination score of 6.98 ($SD = 3.21$) for the sample as a whole.

Figure 1a.

Mean US-expectancy ratings for CS+ and CS- throughout the acquisition phase (trial 1 – trial 12) for the total sample. Vertical bars denote 95% confidence intervals.

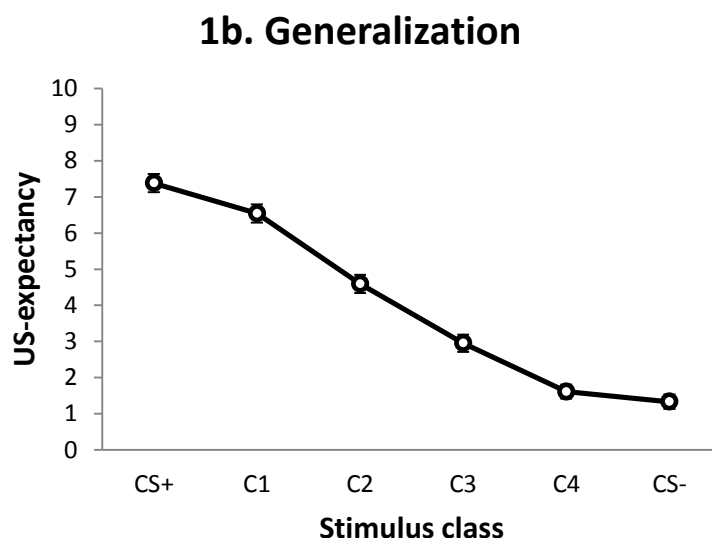


Test for generalization

During the subsequent generalization test phase, US-expectancy ratings were obtained for all six stimulus classes (*Fig. 1b*). The mean US-expectancy ratings were 7.4 (CS+), 6.5 (C1), 4.6 (C2), 2.9 (C3), 1.6 (C4), and 1.3 (CS-). A repeated measures ANOVA with Stimulus class (CS+, C1, C2, C3, C4, CS-) as within subject variable revealed a main effect of Stimulus class, $F(5, 1770) = 526.61$, $p < .001$, partial $\eta^2 = .60$, demonstrating clear generalization of conditioned responding for the sample as a whole. The index of discriminatory aversive learning and responding to the generalization stimuli were associated with each other. Responding to GS Class 1 (i.e., the two GSs most similar to the CS+) was positively correlated with our index of discrimination learning, $r(353) = .31$, $p < .001$, whereas responding to GS class 4 (i.e., the two GSs most similar to the CS-) was negatively correlated, $r(353) = -.50$, $p < .001$.

Figure 1b.

Mean US-expectancy ratings for CS+, CS-, and the four generalization stimulus classes (C1, C2, C3, C4) during the generalization test phase for the total sample. Vertical bars denote 95% confidence intervals.



Prediction of anxiety

Total sample means (and standard deviations) for the questionnaires measuring anxiety at T1 were 2.99 (2.92) for the DASS-A, 39.42 (9.27) for the STAI-T, and 36.0 (9.07) for the STAI-S. At T2, this was 2.58 (3.01) for the DASS-A, 38.16 (9.57) for the STAI-T, and 32.91(8.93) for the STAI-S. Pearson correlations between these measures were all significant ($p < .001$) at and across both time points. In terms of effect sizes, correlations ranged from .33 to .79.

Index of discrimination learning as a predictor

To test whether individual differences in discriminatory aversive learning had predictive value for anxiety complaints six months later, a regression analysis was carried out with DASS-A scores at T2 as the dependent variable and the index of discrimination learning as the independent variable. Results showed that impaired discrimination learning predicted higher DASS-A scores after a six month follow-up period, even after controlling for baseline anxiety (DASS-A) and trait anxiety (STAI-T; Table 1). Although DASS-A scores at T1, but not trait anxiety, significantly predicted DASS-A scores at follow-up, impaired discrimination learning explained unique variance as well. Impaired discrimination learning did not predict DASS-A scores at baseline (T1), $\beta = -.05$, $t(353) = -0.95$, $p = .341$, suggesting that impaired discrimination learning is not a mere reflection of current levels of anxiety, but instead contributes to its development.

Table 1.

Hierarchical regression analysis predicting Time 2 levels of anxiety (DASS-A) from discriminatory aversive learning, baseline anxiety (DASS-A) and trait anxiety (STAI-T)

	β	t	p
Step 1 ($\Delta R^2 = .271$)			
DASS-A (Time 1)	.47	7.35	<.001
STAI-T (Time 1)	.07	1.12	.261
Step 2 ($\Delta R^2 = .014$)			
Discrimination learning	-.12	-2.29	.023

Next, the CS+ and CS- were investigated separately to check whether this discrimination impairment was driven by either enhanced responding to the CS-, or by attenuated responding to the CS+. After statistically controlling for DASS-A scores and trait anxiety at baseline, elevated responding to the CS- on the last three acquisition trials significantly predicted higher DASS-A scores at T2, $\beta = .11$, $t(268) = 2.01$, $p = .045$. Conversely, decreased responding to the CS+ on the last three acquisition trials showed a trend toward significance $\beta = -.09$, $t(268) = -1.81$, $p = .071$.

In contrast, impaired discrimination learning did not predict state anxiety after six months as measured by the STAI-S, $\beta = -.07$, $t(269) = -1.44$, $p = .152$, or state anxiety at baseline, $\beta = -.07$, $t(353) = -1.33$, $p = .183$.

Generalization of conditioned responding as a predictor

To investigate the predictive value of generalization for the development of anxiety complaints, a hierarchical regression analysis was carried out with DASS-A scores at T2 as the dependent variable. As independent variables, baseline anxiety (DASS-A at T1) and trait anxiety (STAI-T) were entered in the first step of the regression model. The four generalization stimulus classes were entered in the second step of the model, and responding to the CS+ and CS- during the generalization test phase was entered last. Results showed that baseline anxiety but not trait anxiety predicted levels of anxiety at follow-up. Moreover, responding to the generalization stimuli also added significantly to the explained variance (Table 2). Examination of the regression coefficients of the individual predictors revealed that only elevated responding to the GSs that resembled the CS- most (Class 4) was significantly associated with higher levels of anxiety.

Again, generalization of conditioned responding was not significantly associated with baseline anxiety (DASS-A at T1), suggesting that generalization predates rather than coincides with current levels of anxiety. Contrarily, none of the four generalization stimulus classes was significantly associated with baseline or follow-up anxiety as measured by the STAI-S (results not shown).

Table 2.

Hierarchical regression analysis predicting Time 2 levels of anxiety (DASS-A) from generalization of conditioned responding, baseline anxiety (DASS-A) and trait anxiety (STAI-T)

	β	t	p
<i>Step 1</i> ($\Delta R^2 = .271$)			
DASS-A (Time 1)	.50	7.81	<.001
STAI-T (Time 1)	.06	1.01	.315
<i>Step 2</i> ($\Delta R^2 = .029$)			
GS Class 1	-.11	-1.84	.066
GS Class 2	-.00	-0.08	.933
GS Class 3	-.07	-1.12	.263
GS Class 4	.21	2.72	.007
<i>Step 3</i> ($\Delta R^2 = .005$)			
CS+	.01	0.23	.816
CS-	-.09	-1.26	.209

Discussion

In the present study, we examined vulnerability factors for anxiety. Specifically, we investigated whether individual differences in discriminatory aversive learning and generalization contributed to the development of anxiety over time. Impaired discriminatory aversive learning predicted higher levels of anxiety after a six-month follow-up period. Further, elevated responding to generalization stimuli that resemble the conditioned safety cue (CS-) was also associated with higher levels of anxiety at follow-up. These findings suggest that individual differences in discrimination learning and generalization are vulnerability factors for the development of anxiety. Evidently, future studies should investigate whether this task can also reveal onset of clinical anxiety.

To our knowledge, this is the first study that investigates whether individual differences in discriminatory aversive learning and generalization can prospectively predict anxiety complaints outside of the scope of posttraumatic stress disorder (PTSD). A limited number of previous studies

have prospectively investigated the development of symptoms related to PTSD in the aftermath of a traumatic event (Guthrie & Bryant, 2006; Lommen, Engelhard, Sijbrandij, van den Hout, & Hermans, 2013; Pole et al., 2009; Sijbrandij, Engelhard, Lommen, Leer, & Baas, 2013). None of these studies, however, used generalization as a predictor of PTSD symptoms, and only Sijbrandij et al. (2013) investigated the predictive value of discrimination learning (but see also Guthrie & Bryant, 2006). Although impaired fear inhibition learning contributed to the persistence of PTSD symptoms in this study, discriminatory aversive learning was not a significant predictor. This inconsistency with present findings may be attributed to differences in the experimental procedure. For instance, the cues used by Sijbrandij and colleagues were different shapes (star, triangle, square) in different colors (blue, black, purple). In our study, the cues were all black circles presented on a white background, differing only in size, which may have rendered our task more difficult. Thus, our study may have been more sensitive to individual differences in discrimination learning.

Interestingly, our index of discriminatory aversive learning was associated with responding to the generalization stimuli. It is possible that this association is an artefact of the experimental procedure, because discrimination learning and generalization were examined within the same learning task. In other words, individuals who failed to differentiate between the CS+ and CS- during acquisition may have lacked discrimination during the subsequent test for generalization, which in turn resulted in diminished responding to stimuli resembling the CS+, and heightened responding to stimuli resembling the CS-. It is also possible, however, that common processes are involved in discriminatory aversive learning and generalization. For instance, deficits in the processing of perceptual information can account for impaired discrimination learning, as well as for increased generalization to stimuli resembling the originally conditioned stimuli (Craske et al., 2009). Future research should be aimed at gaining insight in these underlying processes.

Analyses with state anxiety (STAI-S) as a dependent variable did not yield significant results. In this context, it seems noteworthy that, unlike the STAI-S, the DASS-A measures anxiety

symptoms isolated from depressive symptoms and stress complaints (Brown, Chorpita, Korotitsch, & Barlow, 1997; Henry & Crawford, 2005). In contrast, the STAI, although more commonly used in conditioning research, has been questioned as a pure measure of anxiety symptomatology because it does not seem to allow differentiation between symptoms of anxiety and depression (Bados, Gómez-Benito, & Balaguer, 2010; Grös, Antony, Simms, & McCabe, 2007). Therefore, impaired discriminatory aversive learning and generalization may constitute vulnerability factors for anxiety, but not for depression.

Our prospective findings also fit with the existing literature on aversive conditioning in psychopathology. Several cross-sectional studies that used differential aversive conditioning procedures have revealed elevated responding to the conditioned safety cue (CS-) among anxious individuals, but not necessarily to the conditioned danger cue (CS+). Moreover, a recent prospective study by Craske and colleagues (2012) showed that elevated responding in a safe condition of an instructed threat paradigm predicted the subsequent first onset of anxiety disorders. These findings are in accord with a fear inhibition model proposed by Davis, Falls, & Gewirtz (2000; see also Lissek et al., 2005), who posit that pathological anxiety may result from a deficit in the inhibition of fear responses in the presence of safety cues. It remains unclear, however, whether elevated responding to a safety cue indeed results from deficits in fear inhibition, or instead whether it results from a failure to discriminate between danger and safety cues. In fact, the differential aversive conditioning procedure does not allow one to differentiate between these theoretical accounts. Interestingly, however, the impaired discrimination between the CS+ and the CS- that significantly predicted levels of anxiety in our study, appeared to be driven by both elevated responding to the CS-, and decreased responding to the CS+. Although not mutually exclusive, these findings seem to favor a discriminatory fear learning deficit over a deficit in fear inhibition. Nevertheless, future studies should use paradigms that allow the independent assessment of inhibition deficits versus failures to discriminate.

Results from the test of generalization revealed that elevated responding to stimuli that resembled the CS- predicted levels of anxiety at follow-up. Relatedly, Lommen, Engelhard, and van den Hout (2010) found that individuals high in neuroticism avoided more generalization stimuli relative to their low neuroticism counterparts. Moreover, high neuroticism individuals avoided more GSs that were closer in perceptual similarity to the CS-, which suggests that they use a ‘better safe than sorry’ strategy. Lommen et al. argued that this strategy may be involved in the development and maintenance of anxiety disorders, as it precludes disconfirmation of irrational fears. Similarly, elevated expectancies of an aversive event in the presence of stimuli that resemble the conditioned safety cue may reflect the cognitive correlate of a better safe than sorry strategy.

Some directions for future studies can be given. First, we only used verbal ratings of US-aversiveness and US-expectancy. Although the US-expectancy measure is a valid and valuable measurement method in fear and anxiety research (Boddez et al., 2013), future studies could aim to replicate these findings with physiological measures of fear (fear-potentiated startle, skin conductance responding). Second, the unconditioned stimuli in this study were aversive emotional IAPS pictures. Verbal ratings indicated that the use of these USs led to successful aversive learning, but future studies may aim to replicate these findings with other, physically salient USs, such as an electric stimulation. The use of verbal US-expectancy ratings and IAPS pictures, however, would be desirable for flexible use in the clinic as well as in the community.

In conclusion, this study demonstrated that individual differences in discriminatory aversive learning and generalization contribute to the development of anxiety after a six month follow-up period. These results add to our knowledge of the pathogenesis of anxiety. Moreover, our findings suggest that individuals at risk for anxiety might benefit from discrimination training, promoting appropriate differentiation between threatening and safe stimuli and situations.

References

- Arnaudova, I., Krypotos, A.-M., Effting, M., Boddez, Y., Kindt, M., & Beckers, T. (2013). Individual differences in discriminatory fear learning under conditions of ambiguity: A vulnerability factor for anxiety disorders? *Frontiers in psychology*, 4, 298. doi:10.3389/fpsyg.2013.00298
- Bados, A., Gómez-Benito, J., & Balaguer, G. (2010). The State-Trait Anxiety Inventory, Trait Version: Does It Really Measure Anxiety? *Journal of Personality Assessment*, 92, 560-567. doi:10.1080/00223891.2010.513295
- Beauchaine, T. P. (2009). The role of biomarkers and endophenotypes in prevention and treatment of psychopathological disorders. *Biomarkers in Medicine*, 3, 1–3. doi: 10.2217/17520363.3.1.1
- Beckers, T., Krypotos, A.-M., Boddez, Y., Effting, M., & Kindt, M. (2013). What's wrong with fear conditioning? *Biological psychology*, 92, 90–96. doi:10.1016/j.biopsycho.2011.12.015
- Boddez, Y., Baeyens, F., Luyten, L., Vastenenwegen, D., Hermans, D., & Beckers, T. (2013). Rating data are underrated: Validity of US expectancy in human fear conditioning. *Journal of behavior therapy and experimental psychiatry*, 44, 201–206. doi:10.1016/j.jbtep.2012.08.003
- Brown, T. A., Chorpita, B. F., Korotitsch, W., & Barlow, D.H. (1997). Psychometric properties of the Depression Anxiety Stress Scales (DASS) in clinical samples, *Behaviour Research and Therapy*, 35, 79-89, doi:10.1016/S0005-7967(96)00068-X
- Chan, C. K., & Lovibond, P. F. (1996). Expectancy bias in trait anxiety. *Journal of abnormal psychology*, 105, 637–647. doi: 10.1037/0021-843X.105.4.637
- Craske, M. G., Rauch, S. L., Ursano, R., Prenoveau, J., Pine, D. S., & Zinbarg, R. E. (2009). What is an anxiety disorder? *Depression and anxiety*, 26, 1066–1085. doi:10.1002/da.20633
- Craske, M.G., Wolitzky–Taylor, K. B., Mineka, S., Zinbarg, R., Waters, A. M., Vrshek–Schallhorn, S., ... Ornitz, E. (2012). Elevated responding to safe conditions as a specific risk factor for anxiety versus depressive disorders: Evidence from a longitudinal investigation, *Journal of Abnormal Psychology*, 121, 315-324, doi:10.1037/a0025738

- Davis, M., Falls, W. A., & Gewirtz, J. (2000). Neural systems involved in fear inhibition: extinction and conditioned inhibition. In M. Myslobodsky & I. Weiner (Eds.), *Contemporary issues in modeling psychopathology* (pp. 113–142). Springer US. doi:10.1007/978-1-4757-4860-4_8
- de Beurs, E., van Dyck, R., Marquenie, L. A., Lange, A., & Blonk, R. (2001). De DASS: Een vragenlijst voor het meten van depressie, angst en stress [The DASS: a questionnaire for the measurement of depression, anxiety and stress]. *Gedragstherapie*, 34, 35-53.
- Dyson, R., & Renk, K. (2006). Freshmen adaptation to university life: Depressive symptoms, stress, and coping. *Journal of Clinical Psychology*, 62, 1231-1244. doi: 10.1002/jclp.20295
- Gazendam, F. J., Kamphuis, J. H., & Kindt, M. (2013). Deficient safety learning characterizes high trait anxious individuals. *Biological psychology*, 92, 342–352.
doi:10.1016/j.biopsycho.2012.11.006
- Gefen, D. R. (2010). Adjustment to college: The relationship among family functioning, stress, and coping in non-residential freshmen students (Doctoral dissertation, City University of New York). Retrieved from <http://gradworks.umi.com/34/26/3426745.html>
- Grillon, C., & Morgan III, C. A. (1999). Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. *J. Abnorm. Psych.*, 108, 134-142. doi: 10.1037/0021-843X.108.1.134
- Grös, D. F., Antony, M. M., Simms, L. J., & McCabe, R. E. (2007). Psychometric properties of the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA): comparison to the State-Trait Anxiety Inventory (STAI). *Psychological assessment*, 19, 369–381. doi:10.1037/1040-3590.19.4.369
- Grühn, D., & Scheibe, S. (2008). Age-related differences in valence and arousal ratings of pictures from the International Affective Picture System (IAPS): Do ratings become more extreme with age? *Behavior Research Methods*, 40, 512-521. doi: 10.3758/BRM.40.2.512

- Guthrie, R. M., & Bryant, R. A. (2006). Extinction learning before trauma and subsequent posttraumatic stress. *Psychosomatic medicine*, 68, 307–311.
doi:10.1097/01.psy.0000208629.67653.cc
- Haddad, A. D. M., Pritchett, D., Lissek, S., & Lau, J. Y. F. (2012). Trait anxiety and fear responses to safety cues: Stimulus generalization or sensitization? *Journal of Psychopathology and Behavioral Assessment*, 34, 323–331. doi:10.1007/s10862-012-9284-7
- Henry, J. D., & Crawford, J. R. (2005). The short-form version of the Depression Anxiety Stress Scales (DASS-21): Construct validity and normative data in a large non-clinical sample. *The British journal of clinical psychology*, 44, 227–239. doi:10.1348/014466505X29657
- Hermans, D., Baeyens, F., & Vervliet, B. (2013). Generalization of acquired emotional responses. In M. D. Robinson, E. R. Watkins, & E. Harmon-Jones (Eds.), *Handbook of cognition and emotion* (pp. 117-134). New York: Guilford Press.
- Indovina, I., Robbins, T. W., Núñez-Elizalde, A. O., Dunn, B. D., & Bishop, S. J. (2011). Fear-conditioning mechanisms associated with trait vulnerability to anxiety in humans. *Neuron*, 69, 563–571. doi:10.1016/j.neuron.2010.12.034
- Jacobs, R. H., Reinecke, M. A., Gollan, J. K., & Kane, P. (2008). Empirical evidence of cognitive vulnerability for depression among children and adolescents: A cognitive science and developmental perspective. *Clinical Psychology Review*, 28, 759-782,
doi:10.1016/j.cpr.2007.10.006
- Kraemer, H. C., Kazdin, A. E., Offord, D. R., Kessler, R. C., Jensen, P. S., & Kupfer, D. J. (1997). Coming to terms with the terms of risk. *Archives of General Psychiatry*, 54, 337–343.
doi:10.1001/archpsyc.1997.01830160065009
- Lang, P.J., Bradley, M.M., & Cuthbert, B.N. (2008). *International affective picture system (IAPS): Affective ratings of pictures and instruction manual*. Technical Report A-8. University of Florida, Gainesville, FL.

- Lissek, S., Powers, A. S., McClure, E. B., Phelps E. A., Woldehawariat G., Grillon, C., Pine, D. S. (2005). Classical fear-conditioning in the anxiety disorders: a meta-analysis. *Behaviour Research & Therapy*, 43, 1391–1424. doi:10.1016/j.brat.2004.10.007
- Lissek, S., Rabin, S., Heller, R. E., Lukenbaugh, D., Geraci, M., Pine, D. S., & Grillon, C. (2010). Overgeneralization of conditioned fear as a pathogenic marker of panic disorder. *American Journal of Psychiatry*, 167, 47-55. doi:10.1176/appi.ajp.2009.09030410
- Lissek, S., Rabin, S. J., McDowell, D. J., Dvir, S., Bradford, D. E., Geraci, M., ...Grillon, C. (2009). Impaired discriminative fear-conditioning resulting from elevated fear responding to learned safety cues among individuals with panic disorder. *Behaviour Research and Therapy*, 47, 111–118. doi:10.1016/j.brat.2008.10.017
- Lommen, M. J. J., Engelhard, I. M., SijBrandij, M., van den Hout, M. A., & Hermans, D. (2013). Pre-trauma individual differences in extinction learning predict posttraumatic stress. *Behaviour Research and Therapy*, 51, 63-67. doi:10.1016/j.brat.2012.11.004
- Lommen, M. J. J., Engelhard, I. M., & van den Hout, M. A. (2010). Neuroticism and avoidance of ambiguous stimuli: Better safe than sorry? *Personality and Individual Differences*, 49, 1001-1006. doi:10.1016/j.paid.2010.08.012
- Lovibond, P. F., & Lovibond, S. H. (1995). The structure of negative emotional states: Comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behaviour Research and Therapy*, 33, 335–343. doi:10.1016/0005-7967(94)00075-U
- Mineka, S., & Zinbarg, R. (2006). A contemporary learning theory perspective on the etiology of anxiety disorders: It's not what you thought it was. *American Psychologist*, 61, 10-26. doi: 10.1037/0003-066X.61.1.10
- Murray, C. J. L., Vos, T., Lozano, R., Naghavi, M., Flaxman, A. D., Michaud, C., ...Lopez, A. D. (2012). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions,

- 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*, 380, 2197-223. doi:10.1016/S0140-6736(12)61689-4
- O'Driscoll, G. A., & Callahan, B. L. (2010). Smooth pursuit in schizophrenia: A meta-analytic review of research since 1993. *Brain and Cognition*, 68, 359-370, doi:10.1016/j.bandc.2008.08.023.
- Pole, N., Neylan, T. C., Otte, C. O., Henn-Hasse, C., Metzler, T. J., Marmar, C. R. (2009). Prospective prediction of PTSD symptoms using fear potentiated auditory startle responses. *Biological Psychiatry*, 65, 235–240. doi:10.1016/j.biopsych.2008.07.015
- Shin, L. M., & Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology* 35, 169–191. doi:10.1038/npp.2009.83
- Sijbrandij, M., Engelhard, I. M., Lommen, M. J. J., Leer, A., & Baas, J. M. P. (2013). Impaired fear inhibition learning predicts the persistence of symptoms of posttraumatic stress disorder (PTSD). *Journal of Psychiatric Research*, 47, 1991-1997. doi:10.1016/j.jpsychires.2013.09.008
- Spielberger, C. D. (1983). *Manual for the state-trait anxiety inventory (STAI-Form Y)*. Palo Alto, CA: Consulting Psychology Press.
- Spruyt, A., Clarysse, J., Vansteenwegen, D., Baeyens, F., & Hermans, D. (2010). Affect 4.0: A free software package for implementing psychological and psychophysiological experiments. *Experimental Psychology*, 57, 36-45. doi:10.1027/1618-3169/a000005
- van der Ploeg, H. M. (2000). *Handleiding bij de Zelf-Beoordelings Vragenlijst. Een Nederlandstalige bewerking van de Spielberger. State-Trait Anxiety Inventory STAI-DY*. [Test Manual Dutch State Trait Anxiety Inventory]. Lisse: Swets & Zeitlinger.
- Torrents-Rodas, D., Fullana, M. a, Bonillo, A., Caseras, X., Andión, O., & Torrubia, R. (2013). No effect of trait anxiety on differential fear conditioning or fear generalization. *Biological psychology*, 92, 185–190. doi:10.1016/j.biopsycho.2012.10.006

World Health Organization. (2013). *Mental health action plan 2013-2020*. Retrieved from http://www.who.int/mental_health/publications/action_plan/en/

Zvolensky, M. J., Kotov, R., Antipova, A. V, & Schmidt, N. B. (2005). Diathesis stress model for panic-related distress: a test in a Russian epidemiological sample. *Behaviour research and therapy*, 43, 521–532. doi:10.1016/j.brat.2004.09.001

Footnotes

1. The IAPS numbers for the most aversive USs were 1300, 3051, and 3250. For the moderately aversive USs, the IAPS numbers were 3300, 9042, and 9320. The mildest level of US-aversiveness consisted of pictures 3241, 7380, and 9373.

Author's contribution

Bert Lenaert, Bram Vervliet, Koen Schruers, en Dirk Hermans contributed to the study design. Bert Lenaert drafted the paper. Yannick Boddez helped structuring the content of the manuscript. All authors provided critical revisions. Bert Lenaert and James W. Griffith performed the data analysis under the supervision of Dirk Hermans.